

REMARKS

Claims 1-30 are pending. Claims 1-14, 17, 20-25, and 27-30 have been withdrawn from consideration as being drawn to a non-elected invention. Claim 18 has been cancelled. Accordingly, claims 15, 16, 19, and 26, as amended, and new claim 31 are under consideration.

Claims 15, 16, 19, and 26 have been amended to better define the claimed subject matter. Support for these amendments is found in the original claims and throughout the specification. Specifically, claim 15 as amended is supported by original claims 15 and 18 and the specification, for example, at page 3, lines 10-16; at page 19, lines 1-2; and at page 11, lines 10-12. Claim 16 as amended is supported by claim 16 as originally presented and the specification, for example, at page 3, lines 16-19. Support for amendment to claim 19 is found in claim 19 as originally presented and, for example, at page 3, lines 27-29. Support for amendment to claim 26 is found in original claims 15 and 26. Support for newly presented claim 31 is found in original claim 15 and in the specification, for example, in Figure 1 and the brief description thereto; in Table 1, at pages 13-14; and at page 11, lines 10-12. No issue of new matter is introduced by these amendments.

The above amendments are clearly indicated in the attachment entitled "MARKED UP VERSION OF THE CLAIMS."

The Examiner has requested that the specification be reviewed for the presence of minor errors. Accordingly, applicants have attended to a review and amendment of the specification and submit herewith a substitute specification in both clean and marked-up copies. Applicants believe that the amendments to the specification as presented herein are believed to address this request.

The above amendments are clearly indicated in the attachment entitled "MARKED UP COPY OF THE SPECIFICATION." Applicants assert that the substitute specification submitted herewith and entitled "CLEAN COPY OF THE SPECIFICATION" contains no issue of new matter.

The Rejection Under 35 USC § 112, First Paragraph

Claims 15-19 and 26 have been rejected under 35 U.S.C. §112, first paragraph, for allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors were in possession of the claimed

invention at the time the application was filed. In that claim 17 has been withdrawn as being directed to non-elected subject matter, applicants assume that any rejections applied to this claim have been rendered in error. Accordingly, applicants assume that the above rejection under 35 U.S.C. §112, first paragraph, is directed to claims 15-16, 18-19, and 26. Claim 18 has been canceled, thereby mooted any rejection of this claim. The rejection appears to be based on the Examiner's contention that the specification fails to provide an adequate written description for a number of aspects pertaining to the invention. Applicants respectfully disagree with the Examiner's position regarding the alleged deficiencies in the specification.

In brief, the present invention is directed to a method wherein a random display library of retroviruses comprising a plurality of retroviruses, said plurality of retroviruses possessing randomized sequences in a receptor binding domain of the envelope (Env) protein (e.g., a VRA or VRB region), is utilized to identify/isolate retroviruses capable of infecting a mammalian cell (e.g., a human cell). Inasmuch as the invention is directed to identifying retroviruses which have a randomized sequence in their envelope protein that confers the ability to transfer viral nucleic acid to a host cell, the invention, in effect, provides a screening method with which to identify a retrovirus capable of infecting any mammalian cell.

Notably, Examples 1 and 2 of the specification illustrate that the screening methods of the invention have been used successfully to identify retroviral vectors capable of infecting novel host cell targets (i.e., D17 canine osteosarcoma cells and 143B human osteosarcoma cells). Moreover, Example 4 teaches additional host cell targets (e.g., prostate tumor cell lines) suitable for the retrovirus Env library screening method of the invention. Also presented in the specification is a description of a modification to the retrovirus Env random-peptide display library useful in the identification of retroviruses capable of targeting macrophage and T-cell hosts. See Example 6. Furthermore, the present inventors have demonstrated that by introducing a randomized stretch of amino acids into the VRA region of the Env protein, they can alter the host cell specificity of a retrovirus, thereby altering its target host cell range.

Examples 1 and 2 and Figures 4, 8 and 13 of the specification provide significant detail pertaining to the creation of Env protein libraries. The results presented therein describe a particular exemplification of the present invention wherein the randomized region was introduced into the cell-targeting region of the feline leukemia virus subgroup A Env protein. Of note, a skilled artisan could readily analyze sequences of other retroviruses to align Env proteins having similar cell-targeting region sequences to that of the FLV subgroup A Env.

Resultant alignment analyses may be used to delineate similar sites in different retroviruses wherein stretches of randomized residues can be incorporated so as to produce random peptide libraries in the Env proteins of other retroviruses. The disclosure of the present invention and references cited therein, which are incorporated in their entirety, read in the context of general knowledge pertaining to the alignment of sequences, renders the execution of such analyses well within the capabilities of a skilled practitioner. Moreover, in view of the above, a skilled artisan would consider it apparent that the written description is ample and the inventors were in possession of the invention as claimed at the time of filing.

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In accordance with the present invention, the host range infectable by a virus is dependent upon the cell type on which the library is tested for infectivity. Indeed, the libraries are generated to identify/isolate a mutant retrovirus with a desired host range (i.e., the ability to infect the cell type tested). The screening process, wherein infected cells in a population are identified (e.g., by assaying for viral nucleic acid sequences by PCR or virally-mediated cytolytic activity, or selection for the expression of a cell-selection marker), serves as positive indicator that a mutant retrovirus with the ability to infect the cell type tested has been generated. In other words, the desired mutant virus no longer has the same host range as the parental virus, but rather is selected for being capable of infecting the novel cell type used in the library screen.

Moreover, the specification is not directed to screening simply for viral "attachment". It involves viral infection or, more specifically, viral gene transfer, which is a far more complicated process than cellular attachment. The virus must necessarily first be able to attach (or bind) to the targeted cell type. It is well documented, however, (see, for example, Cosset et al. J. Virol. (1995), 69:6314-6322), that binding does not necessarily lead to gene transfer. Subsequent steps in viral infection of host cells include conformational changes in the envelope protein followed by fusion of viral and cellular membranes. Finally, in the case of retroviruses, the retroviral genome is delivered to the host cell nucleus, wherein a double-stranded DNA copy of the single-stranded RNA retroviral genome is integrated into the host-cell genome. After integration of the DNA, the gene for a selectable marker, for example, is expressed. The specification presents guidance relating to the use of such selectable markers and means for selection of cells expressing these markers. See page 11, line 29 over to page 12, line 29. Moreover, techniques directed to the expression of cell-selection markers and selection of cells within a population that express such markers are well known and regularly

practiced by skilled artisans. All of these steps subsequent to virus-cell binding/attachment are dependent in large measure upon compatibility with the isolated envelope mutant. Simple attachment to the host cell does not provide the means to select a mutant retrovirus having the desired characteristic of altered infectivity. Thus, the method of the present invention is directed to screening for a retrovirus capable of transferring its nucleic acid (e.g., a cell-selection marker) to a host cell, rather than to screening for a simple binding or attachment assay.

In one aspect of the invention, a specifically targeted virus of the invention is identified by virtue of the expression of a gene encoding a cell selection marker, which is colinear with the mutated envelope gene. For example, the pRVL vector of Example 1 comprises a G418 resistance marker which is used to select for resistant cells. Drug resistant cell clones are isolatable in accordance with the methods set forth in the present specification and by other techniques commonly used in molecular biology laboratories and well known to those skilled in the art. Techniques for separating one colony of drug resistant cells from another colony of drug-resistant cells using cloning rings or by clonal dilution, for example, are common and widely practiced molecular biology techniques. Other cell selection markers and methods for isolation/separation of singly infected cells are described and referenced in the specification. See, for example, page 12, lines 7-23.

The examples presented in the specification are directed to the use of the envelope protein of FeLV-A since it is currently the most convenient envelope protein to employ for retargeting. As indicated in Figure 2, residues 69, 72 and 137 of 1040A virus can be altered as indicated to switch receptor usage from an amphotropic 4070A to a 10A1 host range. As taught by the specification, randomization of regions surrounding one or more of these residues may also be used to advantage to develop useful library-screening reagents. Notably, the utility of FeLV-A in the library screening experiments is presented as an exemplification of the invention. The techniques used successfully with FeLV-A virus are readily applicable to any other retrovirus, as modified with respect to the selection of an appropriate sequence for randomization in the envelope protein.

Applicants also assert that when ten amino acid residues are randomized using degenerate nucleotide sequence substitutions, as described in the specification, the probability of obtaining a stop codon is approximately 50%. While this percentage could be lowered by biasing against or eliminating stop codons from the possible pool of sequences, a skilled

practitioner would appreciate that a 50% probability of incorporating of a stop codon does not lead to an "unacceptable high likelihood" of premature peptide termination as suggested by the Examiner. A library containing two million random nucleotide sequences, for example, would still encode one million open reading frames despite a 50 % frequency of stop codon termination. Moreover, this frequency of premature peptide termination is experimentally viable as evidenced by the success of the present inventors using this technique. See Examples 1 and 2.

Claim 15 and dependent claims therefrom have been amended to clarify host cells that may be infected with viruses of the invention, the type of viruses utilized, and how a virus, having infected a host cell in a population, is identified/isolated. The claims, as amended, are therefore believed to be directed to subject matter for which the specification presents ample written description.

Accordingly, the Examiner is respectfully requested to reconsider and withdraw the rejection of claims 15-16, 18-19, and 26 under 35 U.S.C. §112, first paragraph.

The Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 15-19 and 26 have been rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. Claim 17 has been withdrawn from consideration and claim 18 has been cancelled, thereby mooting any rejection of these claims. Claims 15-16, 19, and 26 have been amended in accordance with the Examiner's suggestions. Accordingly, applicants believe that the amendments to claims 15-16, 19, and 26 have rendered moot the rejection of these claims under 35 U.S.C. §112, second paragraph.

In view of the above, the Examiner is respectfully requested to reconsider and withdraw the rejection of the instant claims under 35 U.S.C. §112.

The Rejection Under 35 U.S.C. § 102

The Examiner has rejected claims 15-18 and 26 under 35 U.S.C. 102(e) as allegedly anticipated by the disclosure of Nolan et al. (U.S. Patent 6,455,247). Claims 17-18 have been withdrawn or canceled, thereby rendering moot the rejection of these claims. Applicants strenuously disagree with the Examiner's position with regard to the disclosure of Nolan et al. As stated hereinabove, the present invention is directed to a method wherein a random display

library of retroviruses comprising a plurality of retroviruses possessing randomized sequences in a receptor binding domain of an exterior protein, the envelope (Env) protein, is utilized to identify/isolate retroviruses capable of infecting a mammalian cell (e.g. a human cell). Nolan et al. fails to teach such a method wherein a random display library comprised of a plurality of retroviruses into which randomized sequences have been incorporated into a receptor binding domain of an **exterior protein** is utilized to identify/isolate retroviruses capable of infecting a mammalian cell. Thus, the disclosure of the Nolan et al. reference omits a step recited in claim 15 and dependent claims therefrom, and, therefore, fails to anticipate the method of the present invention. In view of the above, applicants respectfully request that the rejection of instant claims 15-16, and 26 under 35 U.S.C. §102(e) as allegedly anticipated by Nolan et al. is inappropriate and should be withdrawn.

The Examiner has rejected claim 16 under 35 U.S.C. §102(b) as allegedly being anticipated by or, in the alternative, under 35 U.S.C. §103(a) as obvious over Russell et al. (U.S. Patent 5,723,287) or Buchholz et al. (Nature Biotechnology).

Applicants respectfully submit that the disclosure of Russell et al. fails to anticipate claim 16 of the present invention. Specifically, Russell et al. do not teach or suggest the method of the present invention wherein random peptides are incorporated into a **receptor binding domain** of the Env protein in a retroviral library. In the absence of such teaching, this reference fails to anticipate the method of the instant invention. Accordingly, applicants respectfully request that the rejection of claim 16 under 35 U.S.C. §102(b) as allegedly anticipated by Russell et al. is improper and should, therefore, be withdrawn.

Moreover, Applicants purport that neither Russell et al. nor Buchholz et al. renders obvious the method of claim 16. Of note, neither reference provides guidance with regard to the incorporation of random peptides into a **receptor binding domain** of the Env protein for the purpose of identifying a mutated retrovirus having altered host cell specificity. In contrast, Russell et al. teach that the non-viral polypeptide is fused to the N-terminus of or within 7 amino acids of the N-terminus of a substantially intact viral glycoprotein on the surface of the particle. Similarly, the Buchholz et al. reference is directed to the incorporation of random peptides into the N-terminus of the Env protein. The present invention is directed to the incorporation of random peptides into a **receptor binding domain** (cell targeting region) of the Env protein, which is located approximately 50 amino acid residues downstream of the N-terminus in the VRA, for example. The incorporation of random sequences into this **receptor**

binding domain interrupts the amino acid sequence of the native Env protein and, depending on the insertion sequence, alters the functional activity of the Env protein to change infectivity properties of the mutated Env protein. Inasmuch as neither Russell et al. nor Buchholz et al. suggest a method wherein random peptide sequences are incorporated into a **receptor binding domain** of the Env protein or teach that such a method could be used to generate mutated retroviral particles having altered host cell specificity, neither reference renders obvious the method of the present invention. Applicants, therefore, submit that the rejection of claim 16 under 35 U.S.C. 103 (a) as obvious over Russell et al. (U.S. Patent 5,723,287) or Buchholz et al. (Nature Biotechnology) is not fairly based and should, therefore, be withdrawn.

The Rejection Under 35 U.S.C. § 103

The Examiner has rejected claims 15-19 under 35 U.S.C. §103 (a) as allegedly being unpatentable over Buchholz et al. (Nature Biotechnology) or Russell et al. (U.S. Patent 5,723,287) in view of Larocca et al. (U.S. Patent 6,451,527). Claims 17-18 have been withdrawn or canceled, thereby rendering moot the rejection of these claims. In brief, the deficiencies of the Buchholz et al. and Russell et al. references, as described hereinabove, are not remedied by the disclosure of Larocca et al.

In U.S. Patent 6,451,527, Larocca et al. teach the use of bacteriophage to deliver genes to mammalian cells. Bacteriophage are generally used to infect bacteria, rather than mammalian cells because the efficiency of bacteriophage mediated gene delivery to mammalian cells is extremely low and the delivered genes are not stably integrated into the host cell genome. Applicants submit that the bacteriophage system described by Larocca et al. is disparate from that of mammalian retroviruses and is used only for screening for binding ligands and not for stable gene delivery into mammalian cells. Moreover, applicants assert that the Examiner's comparison of the two systems is inappropriate in view of the profound differences between bacteriophage and retroviral systems with regard, for example, to physical, biological and methodological dissimilarities. Indeed, prior to the method of the present invention, a strategy for extending the system of Larocca et al. to a mammalian retroviral system was far from obvious. This point is underscored by the knowledge that the disclosure of Larocca et al., which was based on over ten years of previous experience with phage display, does not suggest that the findings relating to bacteriophage are extendable to

any viral system. Thus, the Examiner appears to be interpreting the disclosures of Russell et al., Buchholz et al., and Larocca et al. in light of the disclosure of the present invention. Applicants submit that such an interpretation is predicated on hindsight reconstruction and is, therefore, inappropriate.

Thus, none of the references relied upon by the Examiner (i.e., Buchholz et al., Russell et al., or Larocca et al.) considered either alone or in combination would lead a skilled practitioner to the method of the present invention. In view of the above, Applicant believes that the rejection of instant claims 15, 16, and 19 under 35 U.S.C. 103 (a) as allegedly unpatentable over Buchholz et al. (Nature Biotechnology) or Russell et al. (U.S. Patent 5,723,287) in view of Larocca et al. (U.S. Patent 6,451,527) is inappropriate and respectfully request that the rejection be withdrawn.

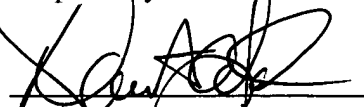
Fees

No additional fees are believed to be necessitated by this amendment. However, should this be an error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment or to credit any overpayment.

Conclusion

It is submitted, therefore, that the claims are in condition for allowance. No new matter has been introduced. Allowance of all claims at an early date is solicited. In the event that there are any questions concerning this amendment, or application in general, the Examiner is respectfully urged to telephone the undersigned so that prosecution of this application may be expedited.

Respectfully submitted,



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Attachments: MARKED UP VERSION OF THE CLAIMS
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CLEAN COPY OF THE SPECIFICATION



MARKED UP VERSION OF CLAIMS

15. (Amended Once) A method of identifying [isolating] a retrovirus [virus that can transfer] capable of transferring its nucleic acid to a host cell, said method comprising the steps of:

(1) administering[,]to a population of host cells, a random display library of viruses comprising a plurality of viruses, wherein each virus differs in relation to other viruses of the plurality as to [the] an amino acid sequence of a receptor binding domain of [the] an exterior protein, wherein said exterior protein is an envelope (Env) protein; [and]

(2) inoculating said population of host cells with said random display library of viruses to infect said population of host cells with said random display library of viruses, wherein infection of said host cell population leads to transfer of retroviral nucleic acid to said host cell population; and

(3) [(2)isolating] identifying a retrovirus [virus] that [infected] transferred its nucleic acid to one of said host cells.

16. (Amended Once) The [A] method of Claim 15 wherein each [member] virus of the plurality encodes [codes, on the same nucleic acid molecule, for both] an Env [exterior] protein of the virus and a cell-selection marker on the same nucleic acid molecule and wherein step [(2)] (3) can be achieved by [cell] selection for virus-infected cells expressing the cell-selection marker.

19. (Amended Once) A method of Claim 15 wherein [the size of] the plurality of retroviruses is more than 1×10^5 .

26. (Amended Once) A retrovirus[, said retrovirus created and isolated by] identified using the method of claim [Claims] 15[, wherein said exterior protein is a retrovirus Env protein or 20].